IODOACETATE POISONING OF THE RAT RETINA*

II. GLYCOLYSIS IN THE POISONED RETINA

BY

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REPEATED intravenous injections of sodium iodoacetate produce a characteristic visual cell degeneration in the monkey, cat, and rabbit (Schubert and Bornschein, 1951; Noell, 1952), the resulting histological picture being very similar to that presented in human retinitis pigmentosa (Noell, 1953). Recently, it has been shown that a similar condition can be produced in the rat by careful injection of two doses of 30 mg. iodoacetate per kg. body weight, an interval of 4 to 5 hrs being allowed between the two injections (Graymore and Tansley, 1958a). Simultaneous administration of sodium malate was shown to decrease the mortality rate of animals treated in this manner, and yet increase the severity of the visual cell damage. In this way it is possible to obtain experimental animals in which the visual cell population is almost completely destroyed.

As early as 1924, Warburg and his associates suggested that all the elements of the retina may not make an equal contribution to the general biochemical picture (Warburg, Posener, and Negelein, 1924) and since then there have been indications that the nerve and receptor components may possess essentially different patterns of metabolism (Noell, 1952; Sjöstrand, 1953; Strominger and Lowry, 1955; Lowry, Roberts, and Lewis, 1956). Noell (1952), on the basis of the differing effects of anoxia and iodoacetate on the electroretinogram of rabbits, believed that iodoacetate acted on some mechanism which was not dependent on oxygen, and suggested that the specific action of this substance on the visual cells might be due to the fact that these cells possessed a predominantly glycolytic form of metabolism.

It was felt that a study of glycolysis in retinæ from rats treated in the manner described above might help to elucidate both the mechanism of action of iodoacetate on the retina as well as certain aspects of the differential metabolism of this tissue. A preliminary report of part of this work has been published elsewhere (Graymore and Tansley, 1958b).

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Materials and Methods

Animals.—The animals used in this investigation were albino rats of either sex, aged 3 to 4 mths and weighing 160–190 g. They were maintained on a standard diet used in this laboratory (Bruce and Parkes, Diet 41, 1946; Parkes, 1946). Both food and water were given *ad lib*.

Solutions for Injection.—Iodoacetic acid was obtained from British Drug Houses Ltd., and was recrystallized from petroleum ether before each series of injections. L-malic acid was obtained from Light and Co. Ltd. Both these substances were adjusted to pH 7.3 with sodium hydroxide immediately before use, and the required dose diluted to 2.0 ml. with saline. Where iodoacetate and malate were administered together, the two concentrated neutralized solutions were mixed in the appropriate proportions and diluted with saline as before. Control animals received an equivalent volume of saline.

Method of Injection.—All solutions were maintained at body temperature and administered intravenously *via* one of the tail veins. Care was taken to avoid very rapid injection, the 2 ml. fluid being administered over 2 minutes. As described in the first paper of this series (Graymore and Tansley, 1958a), 60 mg. iodoacetate per kg. was found to be more effective when administered as a divided dose. In all the experiments recorded below, the higher dosage was administered as two doses of 30 mg./kg. (with or without malate), the injections being separated by a time interval of 4 to 5 hrs; each dose was contained in 2 ml. fluid. The times recorded in the Table represent the interval between the last dose and the killing of the animal. The lower dosage (40 mg./kg.) was given as a single dose.

Histology.—Where histological examination was conducted, the animal was anaesthetized with Nembutal, the descending aorta was ligatured, and the upper part of the body was intravitaly fixed with either Kolmer's or Zenker's fluid. The anterior part of each eye was cut away during dehydration and the posterior part embedded in paraffin wax. Serial sections were cut at 6 μ . Five staining methods were used:

- (1) Haematoxylin and eosin,
- (2) Iron haematoxylin aniline blue-orange G. mixture,
- (3) Mallory's phosphotungstic acid haematoxylin,
- (4) The azan method,
- (5) Feulgen's technique.

Determination of Anaerobic Glycolytic Rate.—The animals were killed by dislocation of the cervical vertebrae, the eyes were enucleated, and the retinae were excised by a special procedure described elsewhere (Graymore, 1958a). There was a delay of 2 min. between the death of the animal and the isolation of both the whole intact retinae. The rate of anaerobic glycolysis was determined by the classical Warburg technique, employing a Krebs–Ringer bicarbonate buffer, gassed with 5 per cent. CO₂–95 per cent. N₂ and fortified with 200 mg. per cent. glucose. The volume of CO₂ liberated from the bicarbonate buffer was taken as an indication of lactic acid production. Preliminary experiments were conducted in which both CO₂ evolution and lactic acid production were measured in the

same flasks and an excellent correlation was obtained. The flasks were gassed for 10 min. and equilibrated for a further 10 min. at 37° C. before taking readings over 60 min. Each flask contained one retina suspended in 4 ml. medium. At the end of the period of incubation, 1 ml. 10 per cent. trichloroacetic acid was added to each flask and the protein was spun down and estimated by the micro-Kjeldahl method. The final result was expressed as the volume of CO₂ in microlitres at N.T.P., liberated in one hr by 1 mg. protein.

This is designated as $q_G^{N_2}(\text{pr.})$.

Results

The Table (opposite) shows the mean anaerobic glycolytic rates of retinæ excised from animals at intervals ranging from 10 min. to 8 weeks after receiving two different doses of iodoacetate with and without malate. Histological examination of the retinæ from those animals treated with the lower dose showed no evidence of cellular degeneration, and the mortality rate was low. The animals receiving the higher dose, on the other hand, showed marked retinal degeneration, and the survival rate was approximately 50 per cent. The normal retina is shown in Fig. 1.

FIG. 1.—Normal rat retina. Kolmer's; iron haematoxylin and phloxine. $\times 300$.

- (1) Pigment epithelium
- (2) Rods
- (3) Outer nuclear layer
- (4) Outer fibre layer
- (5) Inner nuclear layer
- (6) Inner fibre layer
- (7) Ganglion cell layer
- (8) Optic nerve fibre layer

In those treated with 60 mg. iodoacetate/kg. body weight plus malate, the outer nuclear layer was almost completely destroyed over the whole of the retina (Fig. 2, opposite), whereas in those receiving the same dose of iodoacetate but no malate the outer nuclear layer was merely reduced over a large part of the retina (Fig. 3, opposite).

The Table shows that all four treatments resulted in a marked inhibition of glycolysis at 10 min., ranging from an inhibition of 75 per cent. in the case of the higher dose plus malate to one of 66 per cent. for the lower dose without malate, but that in the case of the smaller non-destructive dose, glycolysis had recovered fully by 3 weeks. This was not so in the case of those animals receiving the just sublethal higher dose. In these animals retinal glycolysis increased slightly

FIG. 2.—Rat retina 7 days after receiving 60 mg./kg. iodoacetate as a divided dose. The rods and outer nuclear layer have been damaged, but the latter is not completely destroyed. Kolmer's; Mallory's phosphotungstic acid haematoxylin. $\times 290$.

- (3) Outer nuclear layer
- (4) Outer fibre layer
- (5) Inner nuclear layer
- (6) Inner fibre layer
- (7) Ganglion cell layer
- (8) Optic nerve fibre layer

FIG. 3.—Rat retina 7 days after receiving 60 mg./kg. iodoacetate as a divided dose plus malate. The rods and the outer nuclear layer have virtually disappeared. Kolmer's; Mallory's phosphotungstic acid haematoxylin. $\times 290$.

- (5) Inner nuclear layer
- (6) Inner fibre layer
- (7) Ganglion cell layer
- (8) Optic nerve fibre layer

between 24 hrs and one week but thereafter, up to the 8 weeks of the investigation remained at about half the normal value. Control animals receiving neither malate nor iodoacetate had a $q_G^{N_2}(\text{pr.})$ of 55.3 ± 2.40 .

TABLE

IN VIVO EFFECT OF SODIUM IODOACETATE, WITH OR WITHOUT SODIUM MALATE, ON THE ANAEROBIC GLYCOLYTIC RATE OF THE RAT RETINA

$q_G^{N_2}(\text{pr.})$

Time after Intravenous Injection		10 min.	1 hr	24 hrs	1 wk	3 wks	7-8 wks
Treat-ment	60 mg. iodoacetate/kg. Plus malate	13.75 $\pm$ 0.41 (13)	21.32 $\pm$ 2.87 (9)	18.27 $\pm$ 1.76 (8)	30.15 $\pm$ 0.83 (4)	27.83 $\pm$ 3.92 (6)	26.20 $\pm$ 1.60 (2)
	60 mg. iodoacetate/kg. No malate	13.30 $\pm$ 1.67 (7)	12.70 $\pm$ 0.96 (8)	17.50 $\pm$ 2.23 (4)	—	37.00 $\pm$ 1.54 (3)	—
	40 mg. iodoacetate/kg. Plus malate	16.75 $\pm$ 2.47 (14)	27.10 $\pm$ 1.95 (10)	29.10 $\pm$ 4.42 (4)	41.55 $\pm$ 2.37 (6)	63.25 $\pm$ 1.29 (4)	54.30 $\pm$ 4.11 (5)
	40 mg. iodoacetate/kg. No malate	18.64 $\pm$ 1.47 (9)	40.06 $\pm$ 2.05 (5)	40.60 $\pm$ 3.78 (5)	39.08 $\pm$ 1.84 (6)	—	52.00 $\pm$ 0.50 (2)

Control Values = 55.30 ± 2.43 (6)
Number of determinations in parenthesis

Discussion

The Table indicates that there is little difference between the general inhibitory patterns obtained in the presence and absence of sodium malate, although there may be some difference as regards the actual degree of inhibition produced by a given dose of sodium iodoacetate. In order to avoid undue repetition, most of the discussion will refer to the results obtained when both malate and iodoacetate were administered together. Preliminary experiments showed that sodium malate alone had no effect on retinal glycolysis.

(1) SINGLE DOSE 40MG. IODOACETATE PER KG. BODY WEIGHT PLUS MALATE

The sharp inhibition as early as 10 min. after treatment supports the *in vitro* evidence that iodoacetate is a powerful inhibitor of glycolysis. It is thought to be particularly inhibitory as regards the glycolytic enzyme phosphoglyceraldehyde dehydrogenase (Rapkine, 1938; Cori, Slein, and Cori, 1948). Histological examination confirmed the original finding that this dose does not produce any visible retinal damage (Graymore and Tansley, 1958a), and the Table reveals that the glycolytic rate of these retinæ had returned to normal by the end of the third week. Noell (1951) observed that doses of iodoacetate insufficient to cause permanent visual cell damage could produce an immediate but transient abolition of the electroretinogram, and this phenomenon may well be explained on the basis of the partially reversible early inhibition observed in the present series of results. The point of interest in these values lies in the observation that there is an immediate, marked fall in the glycolytic value, although there is no evidence of cell damage, thus implying that the immediate inhibitory response observed when employing the higher dosage (below) is not secondary to cell death resulting from some other iodoacetate induced cause. It was for this reason that the effect of a non-destructive dose was investigated.

(2) DIVIDED DOSE 60MG. IODOACETATE PER KG. BODY WEIGHT PLUS MALATE

The Table shows that there is an even more severe 10-min. inhibition when this higher dosage is used, although the difference is slight. This is understandable in that the dose of 60 mg. is administered as two distinct injections, separated by a time interval of 4 to 5 hrs, each one of which represents a dose of 30 mg./kg. body weight. The time is taken from the second injection. A comparison of the figures at 1 hr. shows that the marked recovery observed following the lower dose is not apparent after the higher dose has been administered. Between 24 hrs and one week, however, the glycolytic figure is almost doubled. A consideration of the histological changes observed during this time offers an explanation of these fluctuations. The

first signs of pyknotic changes in the visual cells are visible 24 hrs after treatment. After 24 hrs the dead cells are degenerating and by the end of the first week the visual cell debris has been removed. The results presented here are expressed on a total protein basis, so the apparent increase in activity during this latter period could be accounted for in terms of a decreasing protein content rather than a true increase in cellular activity. This tissue loss can be seen from Figs 1 and 3, and was apparent as a reduced average weight of retina.

The final value of 26.2 is approximately half that of the normal retina. This value represents the glycolytic rate of the surviving layers of the retina, *i.e.* the inner nuclear layer and the ganglion cells, and it suggests that these tissues possess a significantly lower glycolytic rate than do the visual cells. If the activity of the visual cells were approximately three times that of the nerve layers, and if the destroyed visual cell population represented approximately half of the total retinal protein, then the glycolytic values at 24 hrs (cells dead but total protein still present) and 7 days (dead tissue removed) could be predicted as being of the order of one quarter and one half of the controls respectively. This is, in fact, what has been found, but the above speculation assumes an even distribution of protein, and should be regarded with caution.

There is, however, another possibility that cannot be excluded. It may be that the metabolism of the surviving nerve layers is inhibited, although not to a sufficient extent to result in histologically obvious damage. In this case the value for the surviving retinae would not be truly indicative of the activity of the normal nerve layer. Since the value remains constant between 1 and 8 weeks, it can be assumed that if there is any inhibition in the inner retina it must be of a permanent nature. Certainly there is no visible manifestation of permanent damage in this part of the retina. The fact that retinae from rats treated with the lower level of iodoacetate have returned to normal after 3 weeks, casts doubt on the possibility that there is some residual, yet temporary inhibition at this stage.

Further evidence that the value of 26.2 represents the true value for the nerve layers is gained from a comparison of values in animals having an inherited retinal degeneration, in which the visual cell components are absent. Again, the glycolytic level of retinae from these animals is about half that of the normal retina (Graymore, Tansley, and Kerly, 1958). The dramatic doubling of the glycolytic activity of the retina of the normal rat towards the end of the second week of life, at a time when the rod cells are undergoing their final differentiation, may be related to the higher glycolytic activity of these elements (Graymore, 1958b, 1958c). The Table shows that, in retinae from animals which were treated with iodoacetate but no malate, the level of glycolysis is higher after 3 weeks than in retinae from animals treated with both these substances. This would seem to reflect the lesser degree of damage noted previously.

The results here presented suggest that the visual cell moiety of the retina possesses a higher glycolytic rate than the nerve and ganglion cell components. The very severe early inhibition of glycolysis may be interpreted as implying that this peculiar effect of iodoacetate is responsible in part for the resulting cellular damage when a just sub-lethal dose is administered, but until more information is obtained regarding the *in vivo* effect of iodoacetate no other aspects of retinal metabolism, such a view must be accepted with caution. The fact that a dose of 40 mg./kg. can produce a marked degree of inhibition over the first hour and yet fail to produce cellular damage, might well mean that there are other important factors which determine the ultimate fate of the visual cells. Such a view is very plausible when the non-specific action of a general SH poison such as iodoacetate is considered. It may be, however, that the higher dose, particularly when divided, exerts its effect by maintaining the inhibition for a sufficient period of time.

In as much as the visual cells appear to have a higher rate of glycolysis and that iodoacetate has been shown to be particularly inhibitory towards this process, the present results do not conflict with the suggestion of Noell (1952) that the specific action of iodoacetate on the visual cells may be due to their peculiar dependence on glycolysis. On the other hand, Lowry, Roberts, and Lewis (1956), using the rabbit and the monkey as experimental animals, have shown that the inner segments of the rods and cones are exceedingly rich in malic dehydrogenase and very poor in lactic dehydrogenase. The former observation is in accord with the observation of Sjöstrand (1953) that the inner segments of the rod cells exhibit dense aggregates of mitochondria. It may be that the visual cells have both a high rate of glycolysis and a high rate of tri-carboxylic acid cycle activity. More information is required before this complex problem is elucidated.

The present results do little to clarify the apparent synergistic action of sodium malate. In the first paper of this series, it was suggested that sodium malate might be converted into pyruvate in the retina, rather than oxaloacetate. It was suggested, further, that if this were so, it could result in a depletion of the available oxidized T.P.N.

The apparent inability of iodoacetic acid completely to inhibit the utilization of glucose suggests that some iodoacetate-insensitive route, such as the Warburg-Dickens shunt, is operating. Depletion of T.P.N. would interfere with this alternative pathway, and thus decrease further the energy production of the iodoacetate-poisoned retina.

It should be stressed, however, that in the present investigations it is difficult to avoid a considerable spread of results. This is due to the difficulties inherent in administering an accurate dosage by injection into the tail vein, as well as a variable animal response as observed previously (Graymore and Tansley, 1958a).

Summary

(1) The effect of iodoacetate on the anaerobic glycolytic rate of the rat retina has been studied.

(2) A dose of iodoacetate plus malate, insufficient to produce permanent histological damage, inhibits glycolysis to the extent of 66 per cent. 10 min. after treatment, but the glycolytic rate has returned to normal within 3 weeks.

(3) The highest tolerated dose of iodoacetate plus malate results in an inhibition of 75 per cent. 10 min. after treatment. Between 1 and 8 weeks, at a time when visual cell degeneration is complete, the glycolytic rate is approximately half that of the controls. This is taken to suggest that the glycolytic activity of the visual cells is higher than that of the remainder of the retina.

(4) The implications of the above findings are discussed.

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